Environmental Health Perspectives
Vol. 84, pp. 183-192, 1990

Quantitative Predictability of Carcinogenicity of the Covalent Binding Index of Chemicals to DNA: Comparison of the *In Vivo* and *In Vitro* Assays

by Maurizio Taningher,* Giovanna Saccomanno,* Leonardo Santi,* Sandro Grilli,† and Silvio Parodi*

The capability of covalent binding to DNA to predict the initiating potential of chemical carcinogens was compared for the assays performed *in vivo* (rodent liver DNA) and *in vitro* (purified DNA incubated in the presence of mouse and rat liver microsomes). A quantitative correlation between DNA adducts and carcinogenic potency was investigated. The *in vivo* assay appeared slightly, but not significantly, more predictive than the *in vitro* assay. Also predictivity was slightly higher both *in vivo* and *in vitro* when we referred to liver carcinogenicity instead of overall carcinogenicity. The predictive ability found for DNA covalent binding (both *in vivo* and *in vitro*) was similar to that of many short-term tests (such as mutagenicity, DNA damage/repair, SCEs, and cell transformation tests).

The covalent DNA binding, measured after incubation with DNA *in vitro* in the presence of liver microsomes, could therfore be a reasonable short-term test offering greater rapidity of execution and requiring the sacrifice of fewer animals than the corresponding *in vivo* test.

Introduction

From our knowledge of the carcinogenicity process, genotoxicity seems to be especially related to initiation. Possibly, other irreversible genomic alterations occurring during progression can also be related to genotoxic effects. Considering that genotoxicity is related only to one or a few steps of the carcinogenicity process, it is not surprising that the correlation between carcinogenicity and genotoxicity is far from perfect.

In addition, when a genotoxicity test is performed, very often important discrepancies are found between the metabolism in the test system and real metabolism in the target cells for the initiation process. Even the end points of genotoxicity tests are not necessarily coincident with events relevant to the initiation process.

*Centro Interuniversitario per la Ricerca sul Cancro, Istituto di Oncologia Clinica e Sperimentale, Università di Genova/Istituto Nazionale per la Ricerca sul Cancro, I-16132 Genova, Italy.

†Centro Interuniversitario per la Ricerca sul Cancro, Istituto di Cancerologia, Università di Bologna, I-40126 Bologna, Italy.

Address reprint requests to M. Taningher, Istituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV, 10, I-16132 Genova Italy.

This relatively weak and partial correlation between the carcinogenic process and genotoxic events is reflected by the results of several studies investigating correlations between carcinogenicity and short-term tests (*1-5*).

In the papers published around 1975 and 1976, that examined very strong genotoxic agents and very clear-cut nongenotoxic compounds, a qualitative correlation around 90% was observed between short-term test results and carcinogenicity (*1-3*). In subsequent papers the average level of correlation was around 70 to 80%. A global picture of the situation is offered from a study performed in 1984 by the International Commission for the Protection Against Environmental Mutagens and Carcinogens (ICPEMC) comparing carcinogenicity and genotoxicity in several short-term tests. In Table 2 of that study, the mean of the accuracy values (correct matchings) for 31 different tests was around 78% (*4*). In a more recent assessment by Tennant et al. (*5*), the level of qualitative correlation (accuracy) was only about 60% for four different *in vitro* short-terms tests. In the data base used for this assessment, the number of compounds suspected of being nongenotoxic carcinogens and/or promoting agents was particularly significant. Other authors (*6-8*) have expressed the opinion that when

the fraction of nongenotoxic chemicals goes up in a data base the correlation between carcinogenicity and short-term tests goes down.

Studies of correlation between carcinogenic potency and quantitative degrees of response for positive short-term test data have given similar results (*9–14*). Again, a correlation is present, but relatively weak. For a summary of the quantitative correlation studies, see Table 2 in our previous paper (*15*). In that paper we also discussed the mathematical relationship between qualitative and quantitative correlation studies (*15*). The fact that a quantitative correlation is always present, even if weak, suggests that the additive role of promotion can attenuate, but not completely eliminate, the quantitative relationship existing between initiating potency and carcinogenic potency, provided that the bulk of the data base analyzed is not made up of pure, nongenotoxic chemicals.

As suggested above, differences between metabolism in the activation system of the short-term test and metabolism in the target cells for the initiation process could be a second major cause of the weak predictablilty of a given short-term test.

In the present study we tried to assess the relevance of metabolic activation *in vivo* in liver cells, versus an *in vitro* system of microsomes, again obtained from liver cells. The amount of DNA adducts was the common end point for both the *in vivo* and the *in vitro* assays considered, so the reason for a possible different degree of predictivity of the two tests had to be essentially related to the different metabolic activation. In addition, two other important factors could decrease the relationship between the *in vivo* and *in vitro* assays: first, DNA repair in the whole liver system may modulate the final DNA binding measured; second, there may be a difference in reactivity between chromosomal DNA and the purified DNA employed in the *in vitro* assay.

We had reason to suspect that a difference could indeed exist, considering the results obtained in several studies which we report below.

For instance, we compared the results obtained by Mirsalis and Butterworth (*16*), who examined the autoradiographic repair induced by dimethylnitrosamine (DMN) in rat liver cells after treatment *in vivo* and also the results obtained by Williams and Laspia (*17*), who examined the autoradiographic repair induced by DMN in primary cultures of hepatocytes where DMN was already clearly active *in vivo* at 10^{-5} mole/kg, but active *in vitro* only at 10^{-2} M concentration.

In a paper by Kerklaan et al. (*18*), the induction of repairable DNA damage in *E. coli* cells evaluated in a host-mediated assay and in an *in vitro* assay were compared. The effects of DMN and 1,2-dimethylhydrazine (1,2-DMH) in *E. coli* cells recovered from the liver of injected mice were already clearly detectable at the dosage of 50 μmole/kg. In the *in vitro* assay (with the presence of mouse liver microsomes), DMN showed an extremely weak (not dose-dependent) response in a range of concentrations between 10^{-2} and 10^{-1} M; 1,2-DMH was completely inactive in the *in vitro* assay up to a concentration of 50 mM.

In our comparison between adduct formation *in vivo* and *in vitro*, the data available were mostly positive. Because the qualitative approach requires a balanced presence of positive and negative results, we were forced to choose a quantitative approach in our correlation study. We already used this methodolgy in several investigations (*9–13*). On the other hand, as a reference point, we had a paper by Lutz presenting a detailed quantitative study of the correlation between carcinogenicity and DNA adduct formation *in vivo (14)*.

Methods

The criteria adopted for the selection and computation of the data and for their correlation studies are discussed in the following section.

Carcinogenic Potency

The carcinogenic potency data were mainly obtained from the data base of Gold et al. (*19*). We can expect a homogenous computation of potencies for all the chemicals listed in it. In this data base the carcinogenic potency is evaluated as TD_{50} and is defined as "that chronic dose rate (in mg/kg body weight/day), which would halve the actuarially adjusted percentage of tumor-free animals at the end of a standard experiment time—the standard lifespan for the species" (*20*). For our computations, the TD_{50} values were normalized in terms of μmole administered per kilogram body weight. For a minor group of chemicals, the above-mentioned data base was integrated with our own data (*21*). Our computations were brought in line with those of Gold et al. (*19*).

For each of the papers listed in the data base, we used the lowest reported TD_{50} value (maximal oncogenic potency) where carcinogenicity was considered statistically significant ($p < 0.05$). When more than one paper was reported in the data base for the same chemical, one TD_{50} value was selected from each of the papers. We then calculated the average of log TD_{50}.

Values of TD_{50} for liver tumors were also considered for correlation studies. The liver was the organ where DNA adducts were measured.

Data were collected only from experiments on mice and rats after oral or parenteral administration. Only positive data were considered.

For our purposes, all the chemicals studied for the correlation between *in vitro* and *in vivo* DNA covalent binding and oncogenic potency had to be genotoxic. We defined as genotoxic the chemicals reported as positive in at least one-third of the genotoxicity tests considered in the data base of PaPajda and Rosenkranz (*22*). This partial arbitrary cut-off point was established in order to exclude a

study that could be suspected as promoting carcinogens more so than initiating carcinogens. One such example could be diethylstilbestrol. We are aware that this cut-off is an arbitrary one. However, it is well known that genotoxic chemicals are not positive in 100% of the tests. For instance, Ashby et al. estimated that nongenotoxic chemicals could be positive in about 20% of the tests, and very genotoxic chemicals could be positive in about 80% of the tests (*23*). Less potent genotoxic agents could give a response between 80 and 20%. The level selected was one considered capable of excluding a significant fraction of nongenotoxic chemicals without being too strict about the genotoxic efficiency required. We were not able to find a more objective threshold. On the other hand, too much time and detailed data was required to analyze the quality of each manuscript and, if necessary, to disagree with the results of the authors about the initiating potential of the chemicals considered.

In Vivo DNA Covalent Binding (*In Vivo*-CBI)

The data concerning the *in vivo* production of DNA adducts were obtained mostly from papers by Lutz (*14*, *24*); some other data were obtained from the papers used for computing the *in vitro* DNA covalent binding index. According to Lutz (*24*), the *in vivo*-CBI was defined as:

$$\frac{\text{micromole chemical bound per mole nucleotides}}{\text{millimole chemical administered per kilogram animal}}$$

The *in vivo*-CBI was computed for experiments on animals sacrificed 4 to 24 hr after treatment. This length of time is probably sufficient, usually being much longer than the biological half-life *in vivo* of most of the chemicals tested. In addition, the temporal range spanned by the different experiments can be considered sufficiently narrow with regard to the scale of CBI potencies. For this reason we did not deem it necessary to modify the *in vivo*-CBI formula proposed by Lutz (*24*).

The *in vivo*-CBI values concerned experiments on mice and rats treated by oral or parenteral routes. Only data on liver DNA adducts were collected. Only data concerning positive results were considered. When numerous data were available for the same chemical, only the first ten values were considered.

In Vitro DNA Covalent Binding (*In Vitro*-CBI)

The (*in vitro*-CBI) was defined as:

$$\frac{\text{micromole chemical bound per mole nucleotides}}{\text{(millimolar chemical in incubation mixture)*(incubation time in minutes)}}$$

The *in vitro*-CBI values were computed from papers cited in the *ICRDB Cancergrams (25)*; data were obtained also from *Medline*, *Toxline*, and *Cancerline* (National Library of Medicine, Bethesda, MD, USA; and Deutsches Institut für Medizinische Dokumentation und Information (DIMDI), Cologne, FRG). When numerous data for the same chemical were available from different papers, only the first ten were used. From a mathematical point of view, it is extremely unlikely that the average of a random sample of 10 values should be totally unrelated to the entire population of all available data. As a consequence, we considered a sample size of 10 data to be adequate. The *in vitro*-CBI values were computed only from experiments in which the metabolic activation was obtained with microsomes prepared from the livers of mice or rats. Different activation systems were not considered. Data for seven directly alkylating chemicals (incubations without microsomes) were also used. The considered experiments used native double-stranded calf thymus or salmon sperm DNA in the incubation mixtures. The molecular weight of the nucleotides was assumed to be equal to 309. In the same paper when more than one *in vitro*-CBI value was computable for the same chemical, only the highest CBI value was used. Only data concerning positive results were considered. Table 1 lists the chemicals for which we computed at least one *in vitro*-CBI value.

Table 1. List of the carcinogens for which an *in vitro*-CBI was computable.[a]

	Liver microsomes				
In vitro-CBI	Animal species[b]	Enzymatic induction[c]	Concentration[d]	Log_{10} (*in vitro*-CBI) mean value	References
Aflatoxin B_1					
1140	R	PB	1.0	2.85	(*26*)
2575	R	WI	e		(*27*)
162	R	PB	83		(*28*)
490	R	PB	1.0		(*29*)
843	R	PB	1.0		(*30*)
1010	R	PB	1.1		(*31*)
2960	R		e		(*32*)
175	R		f		(*33*)
403	R	MC	g		(*34*)
Aflatoxin G_1					
41.9	R	PB	83	1.62	(*28*)
3-Amino-1-methyl-5H-pyrido (4,3-*b*)-indole; (Trp-P-2)					
7.33	R	MC	1.0	0.826	(*35*)
6.13	R	PCB			(*36*)

(Continued on next page)

Table 1. (Continued)

	Liver microsomes				
In vitro-CBI	Animal species[b]	Enzymatic induction[c]	Concentration[d]	Log_{10} (*in vitro*-CBI) mean value	References
Benz(*a*)anthracene					
0.605	R	MC	0.22	-0.218	(*37*)
Benzene					
1.99	M	PB	0.67	0.299	(*38*)
Benzo(a)pyrene					
194	R	MC		1.37	(*39*)
20.1	R	BF	0.50		(*40*)
3.34	R		0.13		(*41*)
49.2	R	MC	g		(*34*)
23.2	R	MC	0.20		(*42*)
1710	R	MC	2.0		(*43*)
40.0	R	MC	h		(*44*)
0.258	R	MC	0.22		(*37*)
3.17	M	WI	0.63		(*45*)
62.6	R	MC	0.36		(*31*)
Bromoacetaldehyde					
181	M		0.62	2.26	(*46*)
Bromobenzene					
5.36	M	PB	0.67	0.729	(*47*)
Bromoethanol					
15.4	M		0.62	1.19	(*46*)
Carbon tetrachloride					
0.110	M	MC	i	-0.959	(*48*)
Chlorobenzene					
2.84	R	PB	0.67	0.453	(*49*)
Cyclophosphamide					
1.22	R	PB	0.25	0.0864	(*50*)
Dibenz(*a,h*)anthracene					
0.192	R	MC	0.22	-0.717	(*37*)
1,2-Dibromo-3-chloropropane					
1.03	R		2.2	0.0128	(*51*)
1,2-Dibromoethane					
6.63	M	PB	0.67	0.587	(*52*)
18.7	M	PB	0.67		(*53*)
8.02	M		0.62		(*46*)
3.30	M		0.62		(*54*)
0.263	R		2.2		(*51*)
3,3′-Dichlorobenzidine					
1.87	R		0.75	0.272	(*55*)
1,1-Dichloroethane					
1.20	M	PB	0.67	0.0792	(*56*)
1,2-Dichloroethane					
8.74	R	PB	0.67	0.591	(*52*)
8.17	M	PB	0.67		(*53*)
0.827	M		0.62		(*54*)
Diethylnitrosamine					
0.552	R	PB	1.6	-0.258	(*57*)
Diethylstilbestrol					
1.33	R	MC	0.80	0.124	(*58*)
15,16-Dihydro-11-methylcyclopenta(*a*)phenanthren-17-one					
24.1	R		j	1.38	(*59*)
7,12-Dimethylbenz(*a*)anthracene					
20.8	R	PCB	0.25	0.711	(*60*)
3.41	R	PB	1.0		(*61*)
3.22	R	MC	0.22		(*37*)
6.01	R	MC	h		(*44*)
2.60	R	PCB	1.0		(*62*)
Dimethylnitrosamine					
11.8	R	WI	1.6	0.792	(*63*)
6.39	M	A	4.0		(*64*)
3.17	R		1.6		(*56*)
Epichlorohydrin					
0.155	R	PB	0.67	-0.810	(*65*)
Ethionine					
0.162	R	MC	i	-0.790	(*66*)
α-Hexachlorocyclohexane					
0.979	M	PB	0.20	-0.00922	(*67*)
γ-Hexachlorocyclohexane					
1.49	M	PB	0.20	0.173	(*67*)
Hexachloroethane					
6.31	M	PB	0.67	0.800	(*68*)

(Continued on next page)

Table 1. (Continued)

In vitro-CBI	Liver microsomes: Animal species[b]	Liver microsomes: Enzymatic induction[c]	Liver microsomes: Concentration[d]	Log_{10} (*in vitro*-CBI) mean value	References
Isophosphamide					
21.9	R	PB	k	1.34	(*69*)
7-Methylbenz(*c*)acridine					
24.7	M	MC	1.5	1.39	(*70*)
3-Methylcholanthrene					
13.6	R	MC	h	1.78	(*44*)
266	R		2.7		(*71*)
Mycophenolic acid					
1.11	R		1	0.0453	(*72*)
N-Nitrosopiperidine					
0.169	R	PB	1.6	-0.772	(*57*)
Pentachloroethane					
12.9	M	PB	0.67	1.11	p
Quinoline					
4.00	R	PCB	1.0	0.602	(*73*)
1,1,2,2-Tetrachloroethane					
2.92	M	PB	0.67	0.465	(*74*)
Tetrachloroethylene					
3.74	R	PB	0.67	0.573	(*75*)
1,1,1-Trichloroethane					
0.675	M	PB	0.67	-0.171	(*76*)
1,1,2-Trichloroethane					
16.4	R	PB	0.67	1.21	(*77*)
Trichloroethylene					
0.316	M	PB	0.58	-0.294	(*78*)
0.817	M	PB	2.0		(*79*)
Diethylsulfate					
0.347				-0.460	(*80*)
Dimethylsulfate					
1.77				0.248	(*81*)
1,1′-Ethylene-*bis*-(1-nitrosourea)					
2.06				0.314	(*82*)
Ethylmethane sulfonate					
0.330				-0.601	(*80*)
0.238					(*83*)
0.200					(*81*)
Ethylnitrosourea					
0.129				-0.321	(*82*)
0.552					(*80*)
1.53					(*81*)
Methylmethane sulfonate					
2.57				0.420	(*83*)
2.69					(*84*)
Methylnitrosourea					
4.12				0.758	(*82*)
2.50					(*85*)
18.3					(*81*)
Semicarbazide					
0.00158				-2.80	(*86*)

[a] *In vitro*-CBI: *in vitro* DNA covalent binding index, as defined in "Methods."
[b] M, mouse; R, rat.
[c] A, acetone; BF, 5,6-benzoflavone; MC, 3-methylcholanthrene; PB, phenobarbital; PCB, polychlorinated biphenyls; WI, without induction.
[d] The concentration is expressed in mg protein/mL incubation mixture, unless otherwise stated.
[e] 0.50 nmole P-450/mL incubation mixture.
[f] 0.25 nmole P-450/mL incubation mixture.
[g-l] Microsomes from: 0.25; 0.7; 0.3; 0.14; 0.25 and 2.0 g of liver incubated in: 5, 6, 6, 3, 3, and 50 mL of incubation mixture, respectively, for footnotes *g, h, i, j, k, l.*
[m] In the presence of cytosolic proteins.
[n] DNA source not specified.
[o] HeLa cell DNA.
[p] Our unpublished data.

Quantitative Correlation Studies

In a previous paper we showed that general sets of values concerning oncogenic potencies or short-term test potencies tend to display approximately a log-normal distribution (*15*). As a consequence, the correlation studies were made between log_{10} of potencies. When more than one potency value was available for the same compound, we used the average of log_{10} potencies. Usually, the clouds of points in graphs of log_{10} of potency X versus log_{10} of potency Y are also compatible with a linear regression analysis and parametric statistics (data not reported).

In order to determine the effects of a possible deviation from a log-normal distribution, the different correlations were also analyzed with the nonparametric Spearman's rank correlation coefficient, as reported in Tables 2 and 3.

Results

As detailed in the "Methods" section, the main conditions adopted for normalizing the data were studies on mice and rats treated by oral or parenteral routes for carcinogenicity data, on mouse and rat liver DNA for *in vivo*-CBI data, and using mouse or rat liver-metabolizing systems for *in vitro*-CBI data.

In these conditions, carcinogenicity data for 49 genotoxic chemicals (as defined in "Methods") and *in vivo*-CBI data for 44 genotoxic chemicals were available. They had *in vitro*-CBI values spanning a range more than five $logs_{10}$ wide, and totaled 48 compounds. However, 19 of them (i.e., chemicals No. 7-11, 17, 20, 21, 26-28, 30, 32, 34, 35, 39, 41, 43, 48) could not be used for any correlation because *in vivo* data were either unavailable or the chemicals were not defined as genotoxic compounds. Thus, we were left with 29 useful compounds. In Table 1 both the *in vitro*-CBI values for each experiment and average log_{10} (*in vitro*-CBI) values were reported; however, for correlation studies only the average log_{10} values were used.

We wanted to consider only genotoxic compounds. The importance of this point may be illustrated by considering that during their lifetime animals subjected to chronic carcinogenicity experiments with promoters can be exposed to a background level of initiating events that are sufficient to combine with the effects of a full-promoting treatment, thus generating a detectable tumor incidence. As a consequence, even purely or prevalently promoting agents can give positive results in chronic carcinogenicity experiments in rodents. However, a study of the correlation between DNA adduct formation and carcinogenicity makes sense only if we deal with initiating agents.

In order to increase the probability that we were dealing with an initiator and not a promoter, we established the condition that a chemical could be used in our correlation study only if it was positive in at least one out of three genotoxicity tests, as judged

Table 2. Quantitative correlations among overall carcinogenicity (TD_{50}), *in vivo* DNA covalent binding (*in vivo*-CBI), and *in vitro* DNA covalent binding (*in vitro*-CBI).[a]

Couple of parameters	No. of chemicals	Correlation coefficient, r[b]	Correlation coefficient per ranks, r_s[d]
Overall correlation			
Log (*in vitro*-CBI) = f (−Log [TD_{50}])	26	0.44	0.30
Log (*in vivo*-CBI) = f (−Log[TD_{50}])	41	0.52	0.51
Log (*in vitro*-CBI) = f (Log [*in vivo*-CBI])	21	0.64	0.53
Correlation for the same 18 chemicals			
Log (*in vitro*-CBI) = f (−Log [TD_{50}])	18	0.42[c]	0.27
Log (*in vivo*-CBI) = f (−Log [TD_{50}])	18	0.46[c]	0.36
Log (*in vitro*-CBI) = f (Log [*in vivo*-CBI])	18	0.58	0.47

[a]The definitions of the parameters are reported in "Methods." *In vitro*-CBI data were obtained from the data base listed in Table 1. *In vivo*-CBI data were obtained from (*14,24,28,33,41,50,53,65,66,68,74-76*); our unpublished data were used for pentachloroethane. TD_{50} were obtained from Gold et al. and Parodi et al. (*19,21*).

[b]Parametric statistical computations according to Snedecor and Cohran (*87*). The r values are statistically different from zero with a $p < 0.05$ except where otherwise specified.

[c]r values for which $p < 0.10$.

[d]Nonparametric statistical computations according to Siegel (*88*). r_s = nonparametric correlation coefficient according to Spearman.

Table 3. Quantitative correlations among liver carcinogenicity (TD_{50}), *in vivo* DNA covalent binding (*in vivo*-CBI), and *in vitro* DNA covalent binding (*in vitro*-CBI)[a].

Couple of parameters	No. of chemicals	Correlation coefficient, r[b]	Correlation coefficient per ranks, r_s[d]
Overall correlation			
Log (*in vitro*-CBI) = f (−Log [TD_{50}])	13	0.64	0.55
Log (*in vivo*-CBI) = f (−Log[TD_{50}])	25	0.57	0.63
Correlation for the same nine chemicals			
Log (*in vitro*-CBI) = f (−Log [TD_{50}])	9	0.66[c]	0.50
Log (*in vivo*-CBI) = f (−Log [TD_{50}])	9	0.75	0.85

[a]The definitions of the parameters are reported in "Methods." *In vitro*-CBI data were obtained from the data base listed in Table 1. *In vivo*-CBI data were obtained from (*14,24,28,33,41,50,53,65,66,68,74-76*); our unpublished data were used for pentachloroethane. TD_{50} were obtained from Gold et al. and Parodi et al. (*19,21*).

[b]Parametric statistical computations according to Snedecor and Cohran (*87*). The r values are statistically different from zero with a $p < 0.05$ except where otherwise specified.

[c]r values for which $p < 0.10$.

[d]Nonparametric statistical computations according to Siegel (*88*). r_s = nonparametric correlation coefficient according to Spearman.

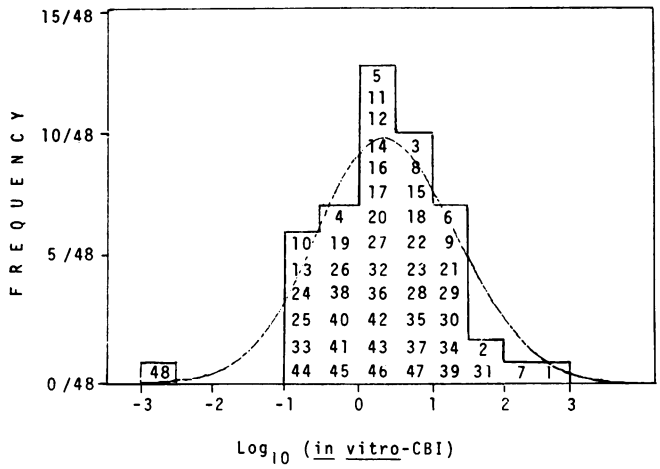

FIGURE 1. Distribution of the $\log_{10}$ (*in vitro*-CBI) mean values referred to the 48 different chemicals listed in Table 1.

from the data base of Rosenkranz and Palajda (*22*).

We analyzed the distribution of the mean values of $\log_{10}$ (*in vitro*-CBI) concerning the 48 chemicals listed in Table 1. As shown by the histogram in Figure 1, these resulting values were approximately log-normally distributed, in accordance with our previous observations (*15*). As a consequence of this result, we considered it acceptable to apply parametric statistics to $\log_{10}$ of potencies.

Table 1 also details the data base of the *in vitro*-CBI values we computed, and gives an idea of the differences in experimental conditions present in the *in vitro* experiments. Although we normalized the results for drug concentration and incubation time, from the point of view of metabolic activation, the experimental conditions were definitely less homogenous than those for the *in vivo* experiments. However, as the reader will see in the following analysis, the additional statistical noise brought about by this lack of data homogeneity apparently did not play an important role. As can be seen in Table 1, for 57 out of the 87 experiments listed, it was possible to normalize the data for microsomal protein concentration. By calculating the correlation between normalized and nonnormalized data, we found $r = 0.87$, suggesting that the lack of this normalization is not terribly disruptive. On the other hand, by using only normalized data, we would have impoverished our data set too much.

With the data we collected, it was possible to study the quantative correlation with carcinogenicity of the *in vitro*-CBI for an overall group of 26 genotoxic chemicals, and the *in vivo*-CBI for an overall group of 41. A correlation study with more homogeneous data was possible with 18 chemicals where triplets of data on carcinogenicity, *in vivo* and *in vitro*-CBI, were available. The results are reported in Table 2.

No statistically significant differences were observed in predictivity of carcinogenicity after discarding from the correlation four directly alkylating chemicals (i.e., chemicals No. 44–47 of Table 1), whose data were available both for *in vitro*-CBI and *in vivo*-CBI.

As previously reported, quantitative correlation levels with carcinogenicity around 0.4–0.5 are very common in most of the short-term tests (*15*). In this respect the results reported in Table 2 seem to suggest that DNA adduct formation can contribute to our knowledge of genotoxicity and the initiating potential of a given chemical with a degree of efficacy similar to that of the most common short-term tests. This conviction is reinforced by the fact that the differences in quantitative predictivity previously found for different short-term tests never reach a level of clear statistical significance (*15*).

In a recent paper, Lutz reported a correlation level with carcinogenicity of the *in vivo*-CBI with $r = 0.81$ (*14*). Compared with our $r = 0.52$, this difference is statistically significant ($p=0.03$). However, it has to be remembered that we considered 12 more chemicals in addition to the 29 considered by Lutz, and we also considered additional experiments for those same 29 chemicals. Moreover, Lutz considered only carcinogenicity results obtained in the same species used for DNA adducts.

The results obtained with the Spearman's test were not very different from those obtained with the parametric approach. The small difference observed is probably related more to the small size of the set considered than to an important systematic deviation from normality of the log of potencies of the general population of data.

As reported in Table 2, we have also investigated the quantitative correlation existing between *in vitro*-CBI and *in vivo*-CBI data. The correlation coefficient with r value around 0.6 found in this study appears relatively high if compared with r values around 0.4, previously found when comparing different short-term tests with one another (*9,10*). This finding confirms that the highest correlation is reached with tests that use the same biological endpoint. As an example, in a previous study, the *in vivo* alkaline DNA elution test, which looks at the endpoint DNA damage, appeared to be correlated with the *in vivo*-CBI by $r = 0.66$. By contrast, it was correlated by lower r values around 0.3, with tests that use different endpoints such as Ames' test and *in vitro* cell transformation test (*9*).

In Table 3 we reported the correlation between DNA adducts and carcinogenicity in the liver. In this case the sample size becomes further reduced. The differences between r values obtained for overall tumors and the r values obtained for liver tumors are not statistically significant. However, a general trend seems to emerge suggesting that the identity of the target organ perhaps plays some role in determining

the correlation level. Even in this case, the predictivities of *in vitro*-CBI and *in vivo*-CBI are very similar.

Discussion

In vivo DNA binding has already been proposed by Lutz (*14*, *24*) as a valid short-term test for assessing genotoxicity.

To our knowledge, this is the first time that *in vivo* and *in vitro* covalent bindings have been compared in terms of their predictivity of carcinogenicity. *In vitro* tests are usually less expensive and more rapid than *in vivo* tests. In addition, the use of *in vitro* tests can significantly reduce the number of animals used to assess the initiating potential of a given chemical as a carcinogen. On the other hand, the general knowledge that we have about absorption, metabolism, and catabolism of chemicals leads us to suspect that *in vivo* assays could be more predictive than assays *in vitro*. As a matter of fact, the papers mentioned in the "Introduction" (*16-18*) seem to suggest that for DMN and 1,2-DMH, two important classical initiating carcinogens, the *in vivo* assay is indeed much better than the *in vitro* assay.

However, this difference is no longer evident when we look at the globality of the 30 to 40 chemicals that we have examined in this work. Admittedly, the sensitivity of the experimental approach adopted for comparing *in vitro* and *in vivo* predictivity is not very great (a larger data base was not available); hence, it remains possible that the *in vivo* assay is slightly more predictive than the *in vitro* assay, as suggested by the small differences in correlation coefficents found in our results. We have to stress that only limited conclusions can be justified by a data base of the size mentioned above.

In a recent study Tennant et al. (*5*) found the qualitative correlation between the carcinogenicity in mice and rats to be only 67%. Given that even the level of this type of internal correlation is limited, we cannot expect especially high correlation levels between short-term tests and carcinogenicity. In this perspective the levels of predictivity found in our study can be considered to be reasonably good.

If we compare the *in vitro*-CBI evaluation (in the presence of liver microsome activation) with other tests, the quantitative predictivity of this assay seems to be similar to that of other classical short-term tests, such as Ames' test, SCEs in mouse bone marrow, morphological transformation *in vitro*, DNA fragmentation *in vivo* (liver), and DNA repair *in vitro* (hepatocytes) (*15*).

In conclusion, *in vitro*-CBI seems to have a predictivity similar to most short-term tests and is not clearly inferior to *in vivo*-CBI. We think that *in vitro*-CBI can be proposed as another short-term test in its own right, especially in the perspective of reducing the number of animals used in toxicity studies.

Our data could also suggest that for other types of short-term tests (mutagenicity and chromosomal damage), *in vivo* versions of the tests are not necessarily dramatically more predictive than the corresponding version *in vitro* (with liver microsome activation). This could be a subject which deserves further investigation.

Finally, it could be interesting to compare *in vitro* and *in vivo*-CBI for a homogeneous class of chemicals. In this respect we have started investigation with the family of chloroethanes.

This work was supported by an ECC contribution (grant EV 4V-0036-I); by grants from CNR Progetto Finalizzato "Oncologia" contracts 87.01566.44 and 87.01315.44; from Ministero della Pubblica Istruzione, Rome; Associazione Italiana per la Ricera sul Cancro, Milan; and Regione Liguria, Genoa, Italy.

We wish to thank Gabriella Frigerio for her careful typing of the manuscript.

REFERENCES

1. McCann, J., Choi, E., Yamasaki, E., and Ames, B. N. Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. (U.S.) 72: 5135-5139 (1975).
2. McCann, J., and Ames, B. N. Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals: discussion. Proc. Natl. Acad. Sci. (U.S.) 73: 950-954 (1976).
3. Sugimura, T., Yahagi, T., Nagao, M., Takeuchi, M., Kawachi, T., Hara, K., Yamasaki, E., Matsushima, T., Hashimoto, Y., and Okada, M. Validity of mutagenicity tests using microbes as a rapid screening method for environmental carcinogens. In: Screening Tests in Chemical Carcinogenesis, Vol. 12 (R. Montesano, H. Bartsch, and L. Tomatis, Eds.), International Agency for Research on Cancer, Lyon, 1976, pp. 81-101.
4. International Commission for Protection Against Environmental Mutagens and Carcinogens. ICPEMC Publication No 9. Report of ICPEMC Task Group 5 on the differentiation between genotoxic and non-genotoxic carcinogens. Mutat. Res. 133: 1-49 (1984).
5. Tennant, R. W., Margolin, B. H., Shelby, M. D., Zeiger, E., Haseman, J. K., Spalding, J., Caspary, W., Resnik, M., Stasiewicz, S., Anderson, B., and Minor, R. Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. Science 236: 933-941 (1987).
6. Rosenkranz, H. S. Foreword (Special Issue: Strategies for the deployment of batteries of short-term tests). Mutat. Res. 205: 1 (1988).
7. International Commission for Protection Against Environmental Mutagens and Carcinogens. ICPEMC Publication No. 16. Testing for mutagens and carcinogens; the role of short-term genotoxicity assays. Mutat. Res. 205: 3-12 (1988).
8. Ramel, C. Short-term testing—are we looking at the wrong endpoints? Mutat. Res. 205: 13-24 (1988).
9. Parodi, S., Taningher, M., and Santi, L. Alkaline elution *in vivo*: fluorimetric analysis in rats. Quantitative predictivity of carcinogenicity, as compared with other short-term tests. In: Indicators of Genotoxic Exposure: Banbury Report No. 13 (B. A. Bridges, B. E. Butterworth, and I. B. Weinstein, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982, pp. 137-155.
10. Parodi, S., Taningher, M., Russo, P., Pala, M., Vecchio, D., Fassina, G., and Santi, L. Quantitative predictivity of the transformation *in vitro* assay compared with the Ames test. J. Toxicol. Environ. Health 12: 483-510 (1983).
11. Parodi, S., Taningher, M., and Santi, L. Induction of preneoplastic nodules: Quantitative predictivity of carcinogenicity. Anticancer Res. 3: 393-400 (1983).
12. Parodi, S., Taningher, M., Zunino, A., Ottaggio, L., De Ferrari, M., and Santi, L. Quantitative predictivity of carcinogenicity

for sister chromatid exchanges *in vivo*. In: Sister Chromatid Exchange: 25 Years of Experimental Research, Part A (R. R. Tice, and A. Hollaender, Eds.), Plenum Press, New York, 1984, pp. 409-429.
13. Bolognesi, C., Taningher, M., Parodi, S., and Santi, L. Quantitative predictivity of carcinogenicity of the autoradiographic repair test (primary hepatocyte cultures) for a group of 80 chemicals belonging to different chemical classes. Environ. Health Perspect. 70: 247-253 (1986).
14. Lutz, W. K. Quantitative evaluation of DNA binding data for risk estimation and for classification of direct and indirect carcinogens. J. Cancer Res. Clin. Oncol. 112: 85-91 (1986).
15. Parodi, S., Taningher, M., and Santi, L. Utilization of the quantitative component of positive and negative results of short-term tests. Mutat. Res. 205: 283-294 (1988).
16. Mirsalis, J. C., and Butterworth, B. E. Detection of unscheduled DNA synthesis in hepatocytes isolated from rats treated with genotoxic agents: an *in vivo-in vitro* assay for potential carcinogens and mutagens. Carcinogenesis 1: 621-25 (1980).
17. Williams, G. M., and Laspia, M. F. The detection of various nitrosamines in the hepatocyte primary culture/DNA repair test. Cancer Lett. 6: 199-206 (1979).
18. Kerklaan, P. R. M., Bouter, S., Van Elburg, P. A., and Mohn, G. R. Evaluation of the DNA-repair host-mediated assay. I. Induction of repairable DNA damage in E. coli cells recovered from liver, spleen, lungs, kidneys, and the bloodstream of mice treated with methylating carcinogens. Mutat. Res. 148: 1-12 (1985).
19. Gold, L. S., Sawyer, C. B., Magaw, R., Backman, G. M., de Veciana, M., Levinson, R., Hooper, N. K., Havender W. R., Bernstein, L., Peto, R., Pike, M. C., and Ames, B. N. A carcinogenic potency database of the standardized results of animal bioassays. Environ. Health Perspect. 58: 9-319 (1984).
20. Peto, R., Pike, M. C., Bernstein, L., Gold, L. S., and Ames, B. N. The TD_{50}: a proposed general convention for the numerical description of the carcinogenic potency of chemicals in chronic-exposure animal experiments. Environ. Health Perspect. 58: 1-8 (1984).
21. Parodi, S., Taningher, M., Ugolini, D., and Santi, L., (Eds.), A database of carcinogenic potencies (problems related to the use of the quantitative component of the information). Istituto Nazionale per la Ricerca sul Cancro, Genva, 1988, p. 279.
22. Palajda, M., and Rosenkranz, H. S. Assembly and preliminary analysis of a genotoxicity data base for predicting carcinogens. Mutat. Res. 153: 79-134 (1985).
23. Ashby, J., de Serres, F. J., Draper, M., Ishidate, M., Jr., Margolin, B. H., Matter, B., and Shelby, M. D. Overview and conclusion of the IPCS collaborative study on *in vitro* assay systems. Progr. Mutat. Res. 5: 117-174 (1985).
24. Lutz, W. K. *In vivo* covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. Mutat. Res. 65: 289-356 (1979).
25. International Cancer Research Data Bank Cancergram Series, CK 11, U.S. Dept. of Health and Human Services Ed. (1979-1985).
26. Guengerich, F. P. Similarity of nuclear and microsomal cytochromes P-450 in the *in vitro* activation of aflatoxin B_1. Biochem. Pharmacol. 28: 2883-2890 (1979).
27. Lotlikar, P. D., Insetta, S. M., and Jhee, E. C. Cytosolic inhibition of liver microsome-mediated binding of aflatoxin B_1 (AFB_1) to DNA. Proc. Am. Assoc. Cancer Res. 21: 78 (1980).
28. Garner, R. C., Martin, C. N., Smith, J. R. L., Coles, B. F., and Tolson, M. R. Comparison of aflatoxin B_1 and aflatoxin G_1 binding to cellular macromolecules *in vitro, in vivo* and after peracid oxidation; characterization of the major nucleic acid adducts. Chem.-Biol. Interact. 26: 57-73 (1979).
29. Bhattacharya, R. K., Firozi, P. F., and Aboobaker, V. S. Factors modulating the formation of DNA adduct by aflatoxin B_1 *in vitro*. Carcinogenesis 5: 1359-1362 (1984).
30. Essigmann, J. M., Croy, R. G., Nadzan, A. M., Busby, W. F., Jr., Reinhold, V. N., Buchi, G., and Wogan, G. N. Structural identification of the major DNA adduct formed by aflatoxin B_1 *in vitro*. Proc. Natl. Acad. Sci. (U.S.) 74: 1870-1874 (1977).
31. Gurtoo, H. L., and Dave, C. V. *In vitro* metabolic conversion of aflatoxins and benzo(a)pyrene to nucleic acid-binding metabolites. Cancer Res. 35: 382-389 (1975).
32. Raj, H. G., Clearfield, M. S., and Lotlikar, P. D. Comparative kinetic studies on aflatoxin B_1-DNA binding and aflatoxin B_1-glutathione conjugation with rat and hamster livers *in vitro*. Carcinogenesis 5: 879-884 (1984).
33. Lotlikar, P. D., Clearfield, M. S., and Jhee, E. C. Effect of butylated hydroxyanisole on *in vivo* and *in vitro* hepatic aflatoxin B_1-DNA binding in rats. Cancer Lett. 24: 241-250 (1984).
34. Alexandrov, K., and Frayssinet, C. Microsome-dependent binding of benzo(a)pyrene and aflatoxin B_1 to DNA, and benzo(a)pyrene binding to aflatoxin-conjugated DNA. Cancer Res. 34: 3289-3295 (1974).
35. Nemoto, N., Kusumi, S., Takayama, S., Nagao, M., and Sugimura, T. Metabolic activation of 3-amino-5H-pyrido (4,3-*b*)indole, a highly mutagenic principle in tryptophan pyrolysate, by rat liver enzymes. Chem.-Biol. Interact. 27: 191-198 (1979).
36. Hashimoto, Y., Takeda, K, Shudo, K., Okamoto, T., Sugimura, T., and Kosuge, T. Rat liver microsome-mediated binding to DNA of 3-amino-1-methyl-5H-pyrido(4,3-*b*) indole, a potent mutagen isolated from tryptophan pyrolysate. Chem.-Biol. Interact. 23: 137-140 (1978).
37. Prodi, G., Grilli, S., Mazzullo, M., Colacci, A., and Arfellini, G. Comparison between photo-induction and microsomal activation of polycyclic hydrocarbons with different oncogenic potency. Toxicol. Pathol. 12: 185-188 (1984).
38. Arfellini, G., Grilli, S., Colacci, A., Mazzullo, M., and Prodi, G. *In vivo* and *in vitro* binding of benzene to nucleic acids and proteins of various rat and mouse organs. Cancer Lett. 28: 159-168 (1985).
39. Ashurst, S. W., and Cohen, G. M. A benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide is the major metabolite involved in the binding of benzo(a)pyrene to DNA in isolated viable rat hepatocytes. Chem.-Biol. Interact. 29: 117-127 (1980).
40. Lubet, R. A., Capdevila, J., and Prough, R. A. The metabolic activation of benzo(a)pyrene and 9-hydroxybenzo(a)pyrene by liver microsomal fractions. Int. J. Cancer 23: 353-357 (1979).
41. Jaggi, W., Lutz, W. K., and Schlatter, Ch. Comparative studies on the covalent binding of the carcinogen benzo(a)pyrene to DNA in various model systems. Experientia 35: 631-632 (1979).
42. Jernstrom, B., Vadi, H., and Orrenius, S. Formation in isolated rat liver microsomes and nuclei of benzo(a)pyrene metabolites that bind to DNA. Cancer Res. 36: 4107-4113 (1976).
43. Hesse, S., Jernstrom, B., Martinez, M., Moldéus, P., Christodoulides, L., and Ketterer, B. Inactivation of DNA binding metabolites of benzo(a)pyrene and benzo(a)pyrene-7,8-dihydrodiol by glutathione and glutathione S-transferases. Carcinogenesis 3: 757-760 (1982).
44. Grilli, S., Rocchi, P., and Prodi, G. Non-enzymatic and microsome-dependent binding of polycyclic hydrocarbons to DNA and polynucleotides. Chem.-Biol. Interact. 11: 351-363 (1975).
45. Ioannou, Y. M., Wilson, A. G. E., and Anderson, M. W. Effect of butylated hydroxyanisole on the metabolism of benzo(a)pyrene and the binding of metabolites to DNA, *in vitro* and *in vivo*, in the forestomach, lung, and liver of mice. Carcinogenesis 3: 739-745 (1982).
46. Banerjee, S., Van Duuren, B. L., and Kline, S. A. Interaction of potential metabolites of the carcinogen ethylene dibromide with protein and DNA *in vitro*. Biochem. Biophys. Res. Commun. 90: 1214-1220 (1979).
47. Colacci, A., Arfellini, G., Mazzullo, M., Prodi, G., and Grilli, S. The covalent binding of bromobenzene with nucleic acids. Toxicol. Pathol. 13: 276-282 (1985).
48. Rocchi, P., Prodi, G., Grilli, S., and Ferreri, A. M. *In vivo* and *in vitro* binding of carbon tetrachloride with nucleic acids and proteins in rat and mouse liver. Int. J. Cancer 11: 419-425 (1973).
49. Grilli, S., Arfellini, G., Colacci, A., Mazzullo, M., and Prodi, G. *In vivo* and *in vitro* covalent binding of chlorobenzene to nucleic acids. Gann 76: 745-751 (1985).

50. Hemminki, K. Binding of metabolites of cyclophosphamide to DNA in a rat liver microsomal system and *in vivo* in mice. Cancer Res. 45: 4237-4243 (1985).
51. Inskeep, P. B., and Guengerich, F. P. Glutathione-mediated binding of dibromoalkanes to DNA: specificity of rat glutathione-S-transferases and dibromoalkane structure. Carcinogenesis 5: 805-808 (1984).
52. Colacci, A., Mazzullo, M., Arfellini, G., Prodi, G., and Grilli, S. *In vitro* microsome and cytosol-mediated binding of 1,2-dichloroethane and 1,2-dibromoethane with DNA. Cell Biol. Toxicol. 1: 45-55 (1985).
53. Arfellini, G., Bartoli, A., Colacci, S., Mazullo, M., Galli, M. C., Prodi, G., and Grilli, S. *In vivo* and *in vitro* binding of 1,2-dibromoethane and 1,2-dichloroethane to macromolecules in rat and mouse organs. J. Cancer Res. Clin. Oncol. 108: 204-213 (1984).
54. Banerjee, S., and Van Duuren, B. L. Binding of carcinogenic halogenated hydrocarbons to cell macromolecules. J. Natl. Cancer Inst. 63: 707-711 (1979).
55. Bratcher, S. C., and Sikka, H. C. Binding of 3, 3'-dichlorobenzidine to DNA and polyribonucleotides *in vitro*. Chem.-Biol. Interact. 38: 369-375 (1982).
56. Colacci, A., Arfellini, G., Mazzullo, M., Prodi, G., and Grilli, S. Genotoxicity of 1,1-dichloroethane. Res. Commun. Chem. Pathol. Pharmacol. 49: 243-255 (1985).
57. Lai, D. Y., Arcos, J. C., and Argus, M. F. Factors influencing the microsome- and mitochondria-catalyzed *in vitro* binding of diethylnitrosamine and *N*-nitrosopiperidine to deoxyribonucleic acid. Biochem. Pharmacol. 28: 3545-3550 (1979).
58. Blackburn, G. M., Thompson, M. H., and King, H. W. S. Binding of diethylstilboestrol to deoxyribonucleic acid by rat liver microsomal fractions *in vitro* and in mouse fetal cells in culture. Biochem. J. 158; 643-646 (1976).
59. Coombs, M. M., Bhatt, T. S., and Vose, C. W. The relationship between metabolism, DNA binding, and carcinogenicity of 15,16-dihydro-11-methylcyclopenta(a)phenanthren-17-one in the presence of a microsomal enzyme inhibitor. Cancer Res. 35: 305-309 (1975).
60. Bigger, C. A. H., Tomaszewski, J. E., Dipple, A., and Lake, R. S. Limitations of metabolic activation systems used with *in vitro* tests for carcinogens. Science 209: 503-505 (1980).
61. Chou, M. W., and Yang, S. K. Identification of four trans-3,4-dihydrodiol metabolites of 7,12-dimethylbenz(a)anthracene and their *in vitro* DNA-binding activities upon further metabolism. Proc. Natl. Acad. Sci. (U.S.) 75: 5466-5470 (1978).
62. Bigger, C. A. H., Tomaszewski, J. E., Andrews, A. W., and Dipple, A. Evaluation of metabolic activation of 7,12-dimethylbenz(a)anthracene *in vitro* by Aroclor 1254-induced rat liver S-9 fraction. Cancer Res. 40: 655-661 (1980).
63. Lai, D. Y., Myers, S. C., Woo, Y.-T., Greene, E. J., Friedman, M. A., Argus, M. F., and Arcos, J. C. Role of dimethylnitrosamine-demethylase in the metabolic activation of dimethylnitrosamine. Chem.-Biol. Interact. 28: 107-126 (1979).
64. Sipes, I. G., Slocumb, M. L., and Holzman, G. Stimulation of microsomal dimethylnitrosamine-*N*-demethylase by pretreatment of mice with acetone. Chem.-Biol. Interact. 21: 155-166 (1978).
65. Mazzullo, M., Colacci, A., Grilli, S., Prodi, G., and Arfellini, G. *In vivo* and *in vitro* binding of epichlorohydrin to nucleic acids. Cancer Lett. 23: 81-90 (1984).
66. Grilli, S., Ferreri, A. M., Rocchi, P., and Prodi, G. *In vivo* and *in vitro* binding of ethionine with nucleic acids. Gann 65: 507-511 (1974).
67. Iverson, F., Ryan, J. J., Lizotte, R., and Hierlihy, S. L. *In vivo* and *in vitro* binding of α- and γ-hexachlorocyclohexane to mouse liver macromolecules. Toxicol. Lett. (Amst.) 20: 331-335 (1984).
68. Lattanzi, C., Colacci, A., Grilli, S., Mazzullo, M., Prodi, G., Taningher, M., and Turina, M. P. Binding of hexachloroethane to biological macromolecules from rat and mouse organs. J. Toxicol. Environ. Health 24: 403-411 (1988).
69. Allen, L. M., and Creaven, P. J. Effect of microsomal activation on interaction between isophosphamide and DNA. J. Pharm. Sci. 61: 2009-2011 (1972).
70. Boux, L. J., and Holder, G. M. The activation of DNA binding of 7-methylbenz(c)acridine catalysed by mouse liver microsomes. Cancer Lett. 25: 333-342 (1985).
71. Eastman, A., and Bresnick, E. Metabolism and DNA binding of 3-methylcholanthrene. Cancer Res. 39: 4316-4321 (1979).
72. Nery, R., and Nice, E. The metabolism and binding of (^{14}C) mycophenolic acid in the rat. J. Pharm. Pharmac. 23: 842-847 (1971).
73. Tada, M., Takahashi, K., Kawazoe, Y., and Ito, N. Binding of quinoline to nucleic acid in a subcellular microsomal system. Chem.-Biol. Interact. 29: 257-266 (1980).
74. Colacci, A., Grilli, S., Lattanzi, C., Prodi, G., Turina, M. P., and Mazzullo, M. The covalent binding of 1,1,2,2-tetrachloroethane to macromolecules of rat and mouse organs. Mutagen. Carcinogen. Teratogen. 7: 465-474 (1987).
75. Mazzullo, M., Grilli, S., Lattanzi, C., Prodi, G., Turina, M. P., and Colacci, A. Evidence of DNA binding activity of perchloroethylene. Res. Commun. Chem. Pathol. Pharmacol. 58: 215-235 (1987).
76. Turina, M. P., Colacci, A., Grilli, S., Mazzullo, M., Prodi, G., and Lattanzi, G. Short-term tests of genotoxicity for 1,1,1-trichloroethane. Res. Commun. Chem. Pathol. Pharmacol. 52: 305-320 (1986).
77. Mazzullo, M., Colacci, A., Grilli, S., Prodi, G., and Arfellini, G. 1,1,2-trichloroethane: evidence of genotoxicity from short-term tests. Gann 77: 532-539 (1986).
78. Banerjee, S., and Van Duuren, B. L. Covalent binding of the carcinogen trichloroethylene to hepatic microsomal proteins and to exogeneous DNA *in vitro*. Cancer Res. 38: 776-780 (1978).
79. Miller, R. E., and Guengerich, F. P. Metabolism of trichloroethylene in isolated hepatocytes, microsomes, and reconstituted enzyme systems containing cytochrome P-450. Cancer Res. 43: 1145-1152 (1983).
80. Sun, L., and Singer, B. The specificity of different classes of ethylating agents toward various sites of HeLa cell DNA *in vitro* and *in vivo*. Biochemistry 14: 1795-1802 (1975).
81. Swenson, D. H., and Lawley, P. D. Alkylation of deoxyribonucleic acid by carcinogens dimethyl sulphate, ethyl methanesulphonate, *N*-ethyl-*N*-nitrosourea and *N*-methyl-*N*-nitrosourea. Biochem. J. 171: 575-587 (1978).
82. Morimoto, K., Tanaka, A., and Yamaha, T. Comparative binding studies on 1,1'-ethylene-bis-(1-nitrosourea) and some 1-alkyl-1-nitrosoureas with nucleic acids and proteins *in vitro*. Gann 70: 693-699 (1979).
83. Bannon, P., and Verly, W. Alkylation of phosphates and stability of phosphate triesters in DNA. Eur. J. Biochem. 31: 103-111 (1972).
84. Lawley, P. D., and Brookes, P. Further studies on the alkylation of nucleic acids and their constituent nucleotides. Biochem. J. 89: 127-138 (1963).
85. Lawley, P. D., Orr, D. J., Shah, S. A., Farmer, P. B., and Jarman, M. Reaction products from *N*-methyl-*N*-nitrosourea and deoxyribonucleic acid containing thymidine residues. Biochem. J. 135: 193-201 (1973).
86. Hayatsu, H., and Ukita, T. The modification of nucleosides and nucleotides. IV. The reaction of semicarbazide with nucleic acids. Biochim. Biophys. Acta 123: 458-470 (1966).
87. Snedecor, G. W., and Cohran, W. G. Statistical Methods, 6th Ed. The Iowa State University Press, Ames, IA 1967.
88. Siegel, S. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York, 1956.